4-12-2010

Cell Adherence and Its Effect on Collagen Expression in Immortalized Rat Chondrocytes (IRC) in Culture

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Abstract

Chondrocytes are cells found in cartilage, and are responsible for the production and secretion of all of the molecules of the extracellular matrix (ECM). Chondrocytes do not survive in the laboratory unless they have been modified in a way that “immortalizes” them. Immortalized chondrocytes serve as a valuable model system in which biomedical research can be carried out without relying on live animal models. Here, we present information on the characterization of the immortalized rat chondrocyte cell line with respect to extracellular matrix production, proliferation rate, optimal culture conditions, the prevelance of apoptotic events, and calcium homeostasis. This work is significant because it will allow us to use this cell line for future studies into the cellular and molecular mechanisms of stress-response of chondrocytes during skeletal development, cancer progression, osteoarthritis, tissue engineering and tissue regeneration. Such studies will depend upon the application of cytochemistry and immunocytochemistry, which are laboratory technique widely used to detect small molecules and proteins within a cultured cell. Antibodies are used to detect the specific proteins via antigen- antibody affinity complex. Fluorescent molecules can be used to visualize the proteins under a microscope. Studies have shown that stress factors on the endoplasmic reticulum such as nutrient depletion, mechanical stress or oxidative stress lead to a decrease in protein expression. Depending on the stress inducer, the cells undergo apoptosis in certain cases. We would like to test how calcium homeostasis is altered and further, how the alteration of calcium homeostasis in chondrocytes affects the expression of collagen II.

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ABSTRACT

Chondrocytes are cells found in cartilage, and are responsible for the production and secretion of all of the molecules of the extracellular matrix (ECM). Chondrocytes do not survive in the laboratory unless they have been modified in a way that "immortalizes" them. Immortalized chondrocytes serve as a valuable model system in which biomedical research can be carried out without relying on live animal models. Here, we present information on the characterization of the immortalized rat chondrocyte cell line with respect to extracellular matrix production, proliferation rate, optimal culture conditions. This work is significant because it will allow us to use this cell line for future studies into the cellular and molecular mechanisms of stress-response of chondrocytes during skeletal development, cancer progression, osteoarthritis, tissue engineering and tissue regeneration. Such studies will depend upon the application of cytochemistry and immunocytochemistry, which are laboratory techniques widely used to detect small molecules and proteins within a cultured cell. Antibodies are used to detect the specific proteins via antigen- antibody affinity complex. Fluorescent molecules can be used to visualize the proteins under a microscope. Alternatively, sodium dodecyl sulfate polyacrylamide gel can be used to detect protein expression. Studies have shown that chondrocytes in vitro show a decrease in collagen II expression. We would like to test how cell adherence alters protein expression in these cells, if at all.

BACKGROUND

- Chondrocytes are important cells of cartilage that express most of the extracellular matrix molecules such as large proteoglycan molecules and collagen proteins including II, IX, and XI.
- 90% of collagen components of the extracellular matrix synthesized by differentiated chondrocytes is collagen II encoded by *col2a1*.
- Immortalization of chondrocytes increases proliferative capacity with retention of chondrocyte phenotype but decreased col2 expression and instead increased collagen XI expression.
- Since IRC cells have a chondrocyte phenotype, they are useful to study the expression of chondrocyte-specific matrix genes, and to examine the effects of environmental factors such as cytokines or growth factors on protein expression.
- In osteoarthritis for example, chondrocytes lose their ability to express collagen II and undergo apoptosis which leads to cartilage loss.
- The goal of my project is to test the effect of IRC adherence or its attachment to the flask on protein expression.
- My hypothesis is that expanded fibroblast- like IRC cells attached to the flask are still capable of expressing collagen II just like round shaped suspended cells.

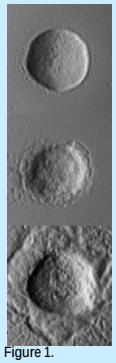

Figure 1. Immortalized rat chondrocytes maintain their round morphology in vitro. Some secretion of extracellular matrix is observed (Wiley Journals).

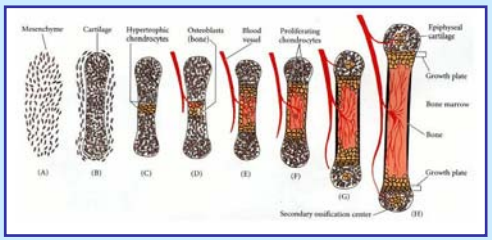

Figure 2. Endochondral Bone Formation. Mesenchymal stem cells differentiate into chondrocytes forming the initial cartilage. Ossification occurs at the initial cartilage longitudinally towards the epiphysis. Any irregularities in the process can affect bone formation.

MATERIALS AND METHODS

Immortalized rat chondrocytes were cultured in an incubator in 5% CO_2 at 37˚C. Cells were grown using cell culture flasks. Four flasks were maintained over four weeks. Four flasks were cultured using two different media. The experiment included cells that were attached to the flask and cells that were suspended. F12/ DMEM was used to treat two flasks; one attached (Fig. 3A) and the other suspended (Fig. 3B). These flasks contained fetal bovine serum (FBS) and thus were nutrient rich and showed fast proliferation. A second medium, OPTI-MEM I was used to treat another set of flasks; one attached (Fig. 3C) and another suspended (Fig 4D). These cells were treated with ascorbic acid to enhance protein expression.

Media was isolated from all four flasks and treated with ammonium sulfate and left overnight. *AccuSpin High Micro 17R* was used to spin the media and Mammalian- Protein Extraction Reagent (M-PER) was used to extract the protein.

5% Sodium Dodecyl Sulfate- PolyAcrylamide Gel Electrophoresis was done to test for protein expression. A picture of the gel was taken and observed for collagen expression. A broad range marker was used to identify the proteins. Coomassie Blue, G250 Stain was used to visualize the bands.

DISCUSSION and CONCLUSION

- Immortalized rat chondrocytes change morphology upon attachment showing expanded fibroblast like shapes
- Clusters are formed, which may be the result of adhering proteins expressed by cells
- Suspended cells did not show any change in morphology
- SDS- PAGE analysis showed some expression of collagen
- Further studies could be done using immunocytochemistry to study the specific proteins expressed by these cells

Acknowledgments

Work in this project is dedicated to Linda M. Mercer, 1949—2010, Oxford Lab Research Technician, Laboratory Manager, Mentor, Lab Mom and Friend.

The project described and continuing work supported by NIH Grant Number P20 RR016454 from the INBRE Program of the National Center for Research Resources and ISBOE Higher Education Research Council Musculoskeletal Research Institute Award.

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RESULTS

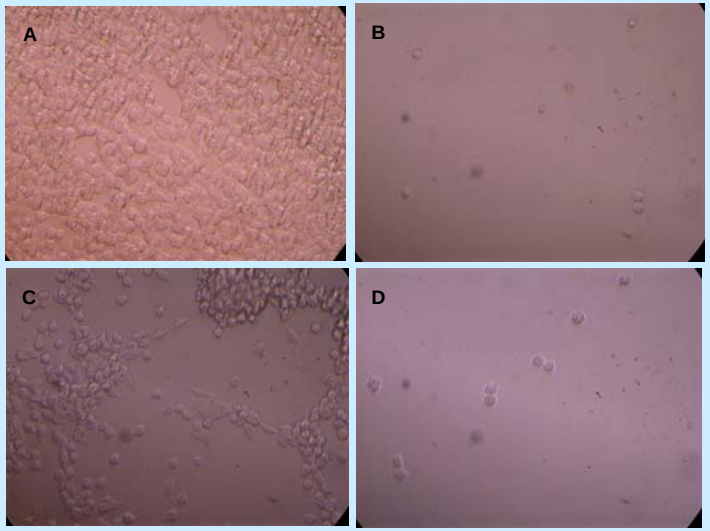

Figure 3. Immortalized rat chondrocytes were cultures using different media and maintained in an incubator in 5% CO_2 at 37˚C. A) IRC cells were grown attached and treated with F12/DMEM (nutrient rich), Streptomycin/ Penicillin. The nutrient rich culture showed rapid proliferation. Clusters were observed B) Suspended IRC treated with F12/DMEM, Strep/Pen., no clusters of more than two cells were observed. C) IRC cultured attached, treated with OPTI-MEM I, Streptomycin/ Penicillin, and ascorbic acid. Attached cells showed change in morphology. They expanded and became fibroblast like. D) Cells suspended in OPTI-MEM I, and treated with ascorbic acid did not change morphology and remained round shaped.

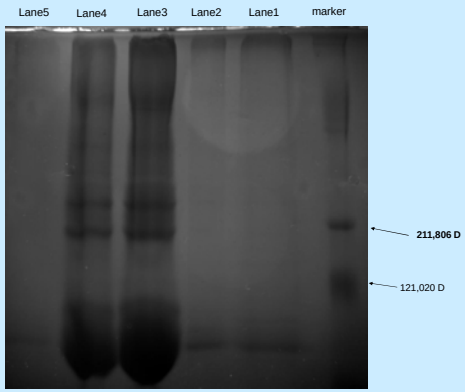

Figure 4. A 5% SDS-GEL was done to test for proteins expressed. Lane 1 was loaded with attached IRC in OPTI-MEM I. Lane 2+3 were both loaded with attached IRC in F12/DMEM. Lane 4 was loaded with suspended IRC in F12/DMEM and lane 5 was loaded with suspended IRC in OPTI-MEM I. Bands were observed at around 211000 Daltons (D) indicating collagen expression by attached IRC in F12/DMEM. Lanes 1, 4 and 5 do not show any obvious bands, which might indicate insufficient protein concentration in sample..